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# EXTRACTION: PRESSURIZED FLUID EXTRACTION

*See* **III / ENVIRONMENTAL APPLICATIONS: Pressurized Fluid Extraction; PRESSURIZED FLUID EXTRACTION: NON-ENVIRONMENTAL APPLICATIONS**

# FATS

## Crystallization

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### Introduction

Fats, as mostly represented by triacylglycerols, are employed in foods, cosmetics, pharmaceuticals, etc. as the main bodies of end products, or as the matrices in which fine chemicals are dispersed. The crystallization behaviour of fats has two major industrial implications: (a) processing of the end products made of fat crystals, such as chocolate, margarine and shortening, whipping cream, etc. and (b) separation of specific fats and lipids materials from natural resources, mostly from vegetable or animal fats and oils, which contain various molecular species having different chemical and physical properties. As for the separation technology of crude fat resources, such as palm oil, milk fats, hydrogenated vegetable oils, etc. it may be worth noting that current market demands raise a great necessity to develop the fractionation of high-melting and low-melting fats and lipids through dry fractionation. The main causes for this are the replacement of hydrogenation by dry fractionation, a new regulation of usage of fat materials for confectionery fats, better functionality of physically refined vegetable oils compared to conventional materials, etc. This review highlights the basic information on recent research on the crystallization of fats, with a specific emphasis on the separation phenomena.

The specific characteristics of the crystallization of fats are, on the one hand, polymorphism, and molecular interactions on the other. No long-chain compound without polymorphic modifications is present, and this property is more enhanced in triacylglycerol (TAG) crystals. The molecular interactions in TAG crystals are operative mostly through van der Waals forces between hydrophobic aliphatic chains, in which geometrical and steric hindrance is critical between the glycerol groups.

### Polymorphism in Fats

Polymorphism is defined as the ability for a chemical compound to form different crystalline or mesophase (liquid crystalline) structures. The melting and crystallization behaviour differs from one polymorph to others, since the different crystal structures correspond to different Gibbs energies.

Polymorphic crystallization is primarily determined by the rate of nucleation, as determined by thermodynamic and kinetic factors. If the first crystallizing form is less stable, it converts to more stable polymorphs through the solid matrix or liquid mediation. As a consequence, morphology of fat crystals is a function of the crystal structure itself, and the thermal processes of the crystallization and sub-

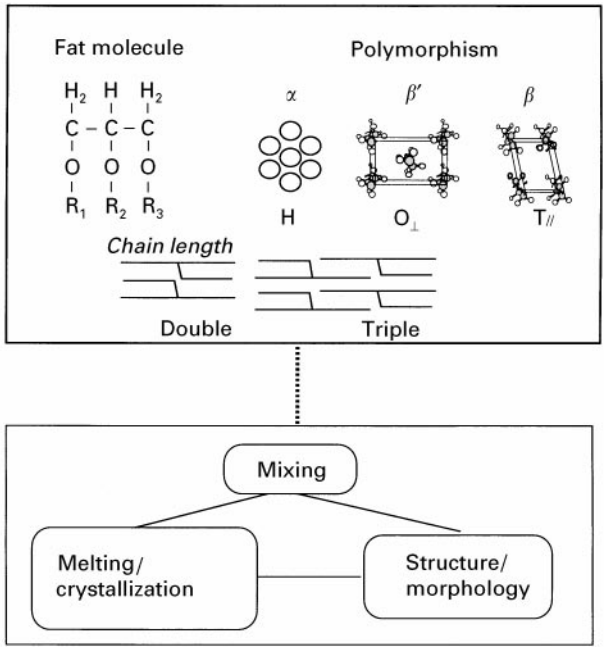


**Figure 1** Relationship of molecular properties of fat crystals and macroscopic physical properties of fats.

sequent transformation. The interrelationships among fat molecule structures, polymorphic crystalline structures, phase behaviour of the mixing of different fats and other ingredients, melting and solidification, and transformation and crystal morphology are illustrated in **Figure 1**.

### Molecule Structures and Polymorphism of Fats

This section briefly describes the molecular structural properties of fat polymorphs.

Typically, three polymorphs, $\alpha$, $\beta'$ and $\beta$, are usually observed in TAG crystals. However, two or even three more forms are present in TAGs if their fatty acid structures are rather complicated, in the sense that the three fatty acid moieties ($R_1$, $R_2$ and $R_3$ in Figure 1) differ in their chemical structures (length, saturated/unsaturated, even-chain or odd-chain, etc.). The three forms are basically characterized by the lateral chain packing mode, expressed in subcell structures: hexagonal (H) packing for $\alpha$, orthorhombic perpendicular ($O_\perp$) packing for $\beta'$, and triclinic parallel ($T_\parallel$) packing for $\beta$. Since the lateral packing mostly determines the crystalline density, it follows that $\alpha$ is the least dense packing, $\beta'$ intermediate and $\beta$ the densest packing. As for the chain length structure, the double chain length is revealed in the TAGs containing similar types of three fatty acid moieties, and, by contrast, the triple chain length structures are revealed in the TAGs where one or two of the fatty acid moieties are largely different in their shapes from the others.

### Polymorphism and Macroscopic Physical Properties

The microscopic properties of the fat polymorphs eventually result in complex macroscopic physical properties of mixing, melting/crystallization and the structure/morphology of the crystallized materials. In particular, the rate of crystallization and the crystal morphology are directly influenced by polymorphism.

**Mixing of fats** The mixing properties of different TAG materials, is highly relevant in fat materials, because many fats present in real systems are multicomponent in two ways: (a) a fat phase containing many different types of TAGs and (b) each TAG molecule involving different types of fatty acid moieties, namely mixed-acid TAGs. Three typical phases occur in binary solid mixtures, when the two components are miscible in all proportions in a liquid state: solid solution phase, eutectic phase, and compound formation. The general tendency in the relationship between the molecular interactions and the phase behaviour may be summarized as follows: structural affinity results in solid solutions, poor interactions form eutectic phases and specific interactions give rise to molecular compounds.

In addition, polymorphism makes the mixing behaviour more complicated. For example, in the mixture of mono-saturated acid TAGs, a eutectic phase with a limited region of solid solution is the stable form when the chain length difference is no larger than two carbon atoms. However, metastable forms of $\alpha$ and $\beta'$ exhibit solid solution phases. Furthermore, the chain length structure also affects the mixing behaviour, even if the polymorphic properties are similar between the component TAGs: for example, the double chain length and triple chain length structures are not miscible with each other.

As for the formation of compound systems, compound formation occurs in particular sets of TAG mixtures, in such mixtures as SOS/SSO (1,2-distearoyl-3-oleoylglycerol) and POP/OPO (P = palmitoyl), where the compound is formed at a concentration ratio around 1 : 1. The mixing behaviour and the crystallization properties in binary fat mixtures will be discussed in a later section.

**Melting behaviour** The melting behaviour in relation to fatty acid composition of TAGs is straightforward: the longer the fatty acid chain, the higher the melting point, and saturated-acid TAGs have higher melting points than unsaturated fatty acid TAGs. The next concern is the effects of polymorphism of fats (which are mostly of a monotropic nature) on the melting and crystallization behaviour. As an example, we take three polymorphic forms of

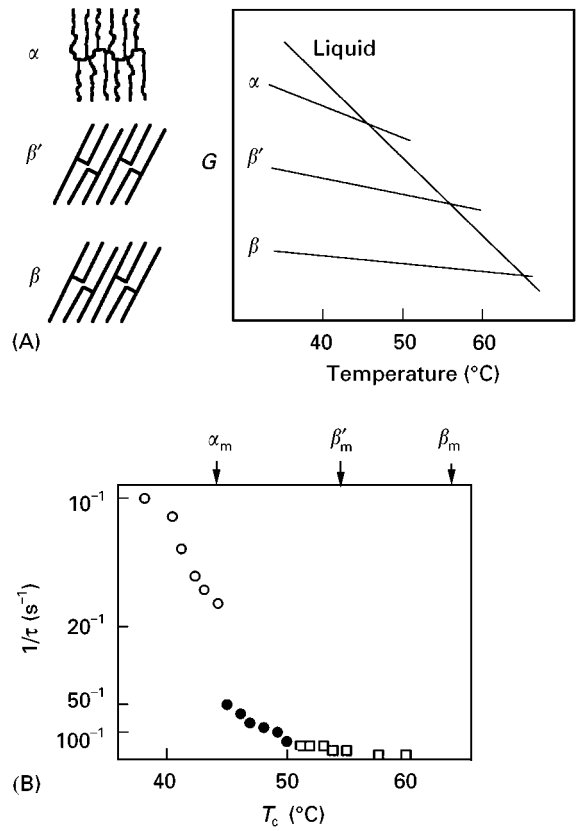


**Figure 2** (A) Structure models and Gibbs energy ($G$)–temperature ($T$) relationship of three polymorphs of PPP, and (B) rate of nucleation of PPP polymorphs. ○, α form; ●, β′ form; □, β form. $T_c$, crystallization temperature.

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tripalmitoylglycerol (PPP), whose structural and crystallization properties have been well elucidated, as illustrated in **Figure 2**.

**Figure 2**(A) shows α, β′ and β forms of PPP. Molecular structures of the three forms are briefly specified by the following: disordered conformation in α, intermediate packing in β′ and most dense packing in β. Therefore, the Gibbs free energy ($G$) values are highest in α, intermediate in β′ and lowest in β, resulting in the lowest melting temperature for α, etc. As a result of the combined effects of the molecular structures and the rate of crystallization, the crystal morphology is of amorphous irregularity for α, tiny bulky shape for β′ and needle shape for β.

The polymorphic properties of PPP are basically common to those in the other fats. However, more complexity is revealed when the fatty acid compositions of the three moieties become heterogeneous, in particular for TAGs involving saturated and unsaturated fatty acid moieties as present in many vegetable fats.

**Structure and morphology** The crystal structures as determined by X-ray analysis using single crystals, are unique for the specific polymorph of a specific TAG. The morphology of crystals, however, is a result of combined interactions of the crystal structure itself, and rate of crystal growth of the specific crystalline faces which are influenced by the growth conditions. For simplicity, however, it may safely be referred to the polymorph-dependent crystal morphology; α reveals irregular shaped tiny crystals, β′ bulky-shaped tiny crystals, and β lozenge-shaped needle crystals.

## Polymorphic Crystallization

Polymorphic crystallization is primarily determined by the rate of nucleation, being governed by thermodynamic and kinetic factors. A primary concern is the polymorphic nucleation, in which the so-called Ostwald step rule is of great interest. This predicts that a phase change may occur step by step by way of successively more stable phases. Thus, the metastable forms nucleate first, prior to the most stable form, when nucleation is induced under large kinetic factors, e.g. supercooling or supersaturation. When the kinetic factors are minimized, the step rule is broken, and the more stable forms are nucleated at very reduced rates.

This tendency was confirmed in the crystallization of three forms of PPP. For PPP, induction time (τ), the time until the occurrence of the first-appearing crystals are detectable, is shortest for α, intermediate for β′ and longest for β as shown in **Figure 2**(B) for the case of simple cooling of the liquid. However, when the temperature of cooling fluctuates around the melting points of α or β′, the nucleation of more stable forms is markedly accelerated, because of melt-mediated crystallization.

Melt-mediated nucleation is ascribed to the monotropic nature of polymorphism, as revealed in the following processes: (a) melting of the less stable form, (b) nucleation and growth of more stable forms, and (c) mass transfer in the liquid formed by melting of the less stable form. It often occurs that the rate of melt-mediated transformation is considerably higher than the rates of crystallization of the stable polymorph without passage through metastable forms. Dynamic X-ray diffraction studied using synchrotron radiation (SR-XRD) has enabled *in situ* observation of the melt-mediated transformation over a time scale of tens of seconds.

In regard to the SR-XRD studies, deeper insights of the polymorphic crystallization have been unveiled by the time-resolved analyses of the crystallization from neat liquid. The result has shown that: (a) the formation of lamella ordering, both for double or triple chain length structures occurs more rapidly

than that of the subcell packing; (b) the rate of the $\alpha$–$\beta'$ melt-mediated transformation is remarkably high when the lamella structures of the pre-existing form ($\alpha$) remains. So-called 'melt-memory effect', meaning the effect of solid structures present in the liquid phase, may be clarified by the time-resolved SR-XRD under temperature variation.

The control of the polymorphic crystallization may be affected by the addition of crystal seeding and the application of shear stresses. As to the former effect, it was found that specific molecular interactions between the crystal seed materials and the polymorphic forms of specific fats are a prerequisite for the seeding effect, in polymorphic correspondence, structural similarity and thermal stability. As a typical demonstration, a quite interesting seeding effect has been observed in cocoa butter crystallization, which has been applied to solidification control of chocolate production processes.

Cocoa butter has six polymorphs of form I through to VI, in which form V is the most desirable. For optimally obtaining form V in the solidification process, a tempering process is applied using the following temperature profile: first cooling the molten chocolate to 26–27°C, reheating to around 30°C, and subsequent cooling for bulk solidification. During the first cooling process, rapid crystallization causes the formation of unstable polymorphic forms of cocoa butter crystals due to Ostwald's Law of stages. The occurrence of the unstable forms are not favourable, because they cause poor moulding and induce fat bloom. Therefore, the first-cooled chocolate is heated to around 30°C, so that the unstable forms melt and quickly change to form V by the melt-mediated transition. After the tempering, chocolate is poured into a mould, and cooled for complete solidification.

The seed crystallization technique aims at achieving two major advantages, compared to the tempering technique: (a) it accelerates the crystallization of form V of cocoa butter, and (b) it does not use the cooling/heating process of tempering. For this purpose, a technique using BOB (1,3-dibehenoyl-2-*sn*-oleoylglycerol) $\beta_2$ polymorph has been developed.

**Figure 3** shows the relative crystallization time ($t_r$) of seeded dark chocolate examined at 30°C. The crystallization time was measured with a rotational viscometer and $t_r$ is defined as the ratio of the crystallization times with and without seeding. Figure 3 clearly shows that the stable form $\beta_1$ of SOS, which is a major component TAG in cocoa butter, is most effective, stable form $\beta_2$ of BOB is in-between and $\beta$ of SSS is least effective. From this result, one may argue that there are three factors in the crystal seeding effect: (a) similarity in the crystal structure between the seed crystal and form V of cocoa butter, (b) thermodynamic stability of the seed crystal, which is related to its solubility in the molten cocoa butter, and (c) the relationship of the acyl chain length of TAG between the seed material and cocoa butter. As to (a), it was shown that the $\beta_2$ of BOB or SOS and form V show identical structural properties to each other. However, the melting point of SOS $\beta$ form, around 43°C, is too low for it to be put into molten cocoa butter at 30°C, and thereby no effect of crystal seeding is operative. As to the $\beta$ form of SSS (tristearoyl-glycerol), its melting point, 70°C, is optimal as far as the thermodynamic stability is concerned. However, the molecular structural affinity between the $\beta$ of SSS and $\beta_2$ of BOB is poorest, because of the difference in the chain length structure: double chain length for $\beta$ of SSS and triple chain length for $\beta_2$ of BOB. Therefore, a final solution of the finest seeding material is the $\beta_2$ of BOB.

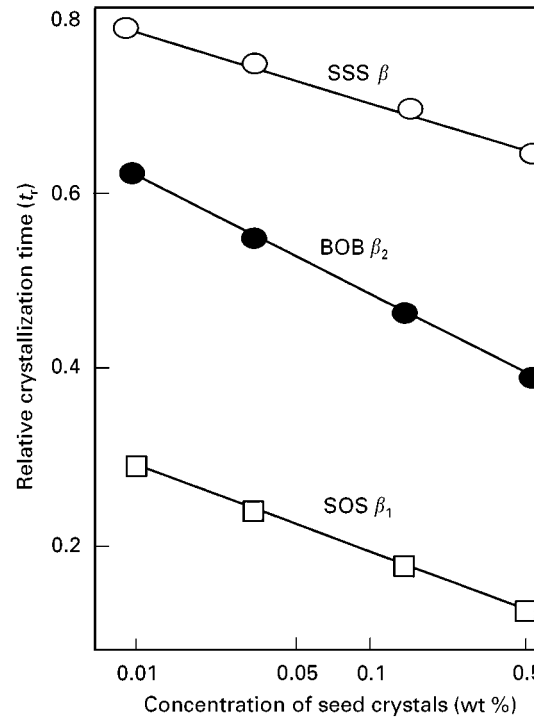


**Figure 3** Relative crystallization time ($t_r$) of cocoa butter with the addition of three crystal seeding materials (SSS, tristearoylglycerol).

As to the shear effect, the newest finding of the shear stress application on cocoa butter crystallization indicates a by-passing effect to crystallize $\beta$-type polymorph (form V), which otherwise does not crystallize directly from the quenched liquid. A time-resolved SR-XRD spectra revealed the transformation from $\beta'$-type to $\beta$-type polymorphs during the initial stages of crystallization under shear stresses. The molecular mechanism for this conversion is not fully understood.

## Phase Behaviour and Crystallization Kinetics in Binary Fat Mixtures

Fats present in natural sources are mixtures of different types of TAGs. Therefore, the complicated behaviour of melting, crystallization and transformation, morphology, etc. of real fat systems is partly due to the physical properties of the component TAGs, and partly or more importantly due to the phase behaviour of the mixtures. To resolve these complexities in mixed systems, a fundamental study has been made on binary and ternary mixtures of specific TAG components. Recent studies carried out on the phase behaviour and crystallization kinetics of the binary mixtures of principal TAGs, are given below.

In general, three typical phases may occur in binary solid mixtures, when the two components are miscible in all proportions in a liquid state: a solid solution phase, a eutectic phase and compound formation. For TAG mixtures, two factors are concurrently dominating: polymorphism and chain–chain interactions.

The polymorphic influence is clearly seen for the PPP–SSS mixture system, where the carbon numbers ($n_c$) of the fatty acid moieties of the two TAGs differ by two. Solid solutions are formed in the $\alpha$ and $\beta'$ forms, yet the eutectic phase was formed in the most stable $\beta$ form. This contrast is ascribed to the less condensed molecular packing in $\alpha$ and $\beta'$ and to the most dense packing for $\beta$. It is reported, however, that the formation of the solid solution in the metastable forms is hindered when the difference in $n_c$ between the component TAGs exceeds four, and that eutectic phases become predominant.

It has recently been found that the phenomena observed in the PPP–SSS mixture above does not simply apply to the mixtures of saturated–unsaturated mixed-acid TAGs such as POP–OPO, POP–PPO and many other similar mixtures, where O is oleic acid moiety, and PPO, for example, is a stereoisomer of POP where the fatty acids are connected to different glycerol carbon atoms. Despite the quite diversified phase behaviour exhibited in these combinations, one may make two important points: (a) steric hindrance between the saturated acid and oleic acid moieties induces the formation of eutectic phases for the $\alpha$, $\beta'$ and $\beta$ polymorphs for the mixture of POP–PPP, and (b) attractive interactions through the saturated and oleic acid moieties cause the formation of molecular compound at a 50 : 50 concentration ratio of the two components. Quite interestingly, the chain length structure of the molecular compound is largely deformed from those of the component molecules, e.g. a triple chain length structure in POP and PPO, but a double chain length structure in the molecular compound of POP–PPO. Therefore, the eutectic phase is revealed in the component and compound materials.

The formation of the molecular compound has two implications for the fat separation problems: firstly, no easy separation is possible by dry fractionation for the molecular-compound mixtures, and secondly polymorphic transformation is affected by the presence of the molecular compounds. More interestingly, the rate of nucleation of the molecular compound is much higher than those of the component TAGs.

The diverse properties in fat mixing behaviour, as illustrated above for a typical model substance, might also be revealed in natural fats having high melting fractions, such as palm oil, shea fats, etc. Therefore, for the purpose of the separation of the high-melting and low-melting fractions, it is pertinent to examine physical analyses of the mixing behaviour of the principal TAG components, as basic research on the one hand, and to extend the main results to more multicomponent systems tightly mimicking real systems.

**See Colour Plate 84.**

*See also:* **II / Crystallization:** Melt Crystallization; Polymorphism; Control of Crystallizers and Dynamic Behaviour.

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